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Larval morphology and life history of *Eutrichosoma mirabile* Ashmead and description of a new species of *Eutrichosoma* (Hymenoptera, Chalcidoidea)

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Abstract

The larval morphology and life history of the weevil parasitoid *Eutrichosoma mirabile* Ashmead (Hymenoptera, Chalcidoidea, Pteromalidae) are described, and the phylogenetic placement of the subfamily Eutrichosomatinae within Chalcidoidea is determined using larval morphological characters. A description of *Eutrichosoma burksi* **sp. nov.** and key to the species of *Eutrichosoma* are provided.

Keywords

Eutrichosomatinae, larval morphology, planidia, Planidial Larva Clade, weevil parasitoid

Introduction

Eutrichosoma is a widespread, but infrequently collected, genus of weevil parasitoids in the subfamily Eutrichosomatinae (Hymenoptera, Chalcidoidea, Pteromalidae). Three species are known: *Eutrichosoma mirabile* Ashmead found throughout North America and Brazil, *E. flabellatum* Bouček from Brazil, and *E. burksi* sp. nov. from California, USA. The natural history is only known for *E. mirabile*, which parasitizes seed-feeding weevil larvae in the genera *Auleutes* and *Smicronyx* (Curculionidae) found on *Parthenium* and *Helianthus* (Asteraceae) (Crawford 1908; Bouček 1974; Charlet and Seiler 1994). *Auleutes* (Ceutorhynchinae) is typically associated with plants in the family Onagraceae and *Smicronyx* (Curculioninae) is typically associated with plants in the

families Asteraceae and Convolvulaceae, however, the plant hosts for the weevils and the weevil hosts for the wasps are likely broader than what is currently known.

Eutrichosomatinae includes two other monotypic genera: *Peckianus laevis* Provancher, a parasitoid of *Apion* (Brentidae) (Bouček and Heydon 1997), ranging from Canada to Brazil; and *Collessina pachyneura* Bouček from Australia, whose natural history is unknown. The taxonomic placement of this group was originally in Cleonyminae (Cleonymidae) by Ashmead (1899), who later placed it within Tanaostigmini (Encyrtidae) when he first provided the species name *Eutrichosoma mirabile* without a description (Ashmead 1904). It was first published as its own family, Eutrichosomatidae, by Peck (1951) and then reclassified as the subfamily Eutrichosomatinae within Pteromalidae by Bouček (1974). The digitate labral morphology of adult *E. mirabile* was regarded as similar to that of Eucharitidae and Perilampidae but independently derived (Darling 1988); however, the phylogenetic placement of this group based on a combination of morphological and molecular data suggests that Eutrichosomatinae belong within the Perilampidae+Eucharitidae clade (Heraty et al. 2013), which we here term the 'Planidial Larva Clade' (PLC) for their highly derived first-instar larvae.

Planidia, which means "diminutive wanderers," are hypermetamorphic, active first-instar larvae (Clausen 1940a). The eggs are often laid away from the host, which requires the planidia to be adapted for free-living, being more mobile and more sclerotized than typical parasitoid larvae and showing host-seeking behavior. Planidia undergo metamorphosis between the first and second instar, with the latter typically resembling sessile, non-planidial larvae that, within Hymenoptera, are termed hymenopteriform (Clausen 1940a; Pinto 2009). Within the PLC, planidia first attach externally to the host larva and begin further development through later instars on the host pupa (Clausen 1940b; Darling and Miller 1991; Darling 1992; Darling 1999). Until the host pupates, the planidia need to be mobile and able to find/recognize their hosts so that they can detach and reattach when their hosts molt. There are several groups of Diptera that have planidial first-instar larvae (Acroceridae, Nemestrinidae, Bombyliidae, Tachinidae, Asilidae) as well as Hymenoptera (Eucharitidae, Perilampidae, *Euceros* in Ichneumonidae), with multiple origins of planidial larvae present in both orders. We predict a single origin for planidial larvae in Chalcidoidea, and thus, if part of the PLC, the first-instar larvae of *Eutrichosoma mirabile* should have planidial morphology or otherwise some transitional form. We were able to collect and study the larvae of *E. mirabile* in Arizona to better understand their life history, and we provide support for the phylogenetic placement within the PLC based on larval morphology. In the process of examining museum material, we discovered a distinctive new species from California.

Materials and methods

Eutrichosoma mirabile was collected at a field site close to Portal, Arizona, along Foothills Rd (31.952N, 109.139W) in August 2016, 2018, and September 2018.

Adult *Eutrichosoma mirabile* were swept off of whitethorn acacia (*Vachellia constricta* (Benth.) Seigler & Ebinger; Fabaceae). Seedpods of whitethorn acacia were collected and individually dissected in lab. Larvae of hosts and parasitoids were collected into 95% ethanol. Voucher specimens were assigned individual plasticized barcodes and deposited in the Entomology Research Museum at the University of California Riverside (**UCRC**). For slide mounting, larvae were cleared with 10% KOH, and then slide mounted in Hoyer's medium; after drying, slides were sealed with clear nail polish. Some larval specimens were retained in 95% ethanol. For scanning electron microscopy (SEM), larvae were dried using hexamethyldisilazane (Heraty and Hawks 1998), mounted on double sided sellotape, and sputter coated with palladium. SEM images were taken at the University of California Riverside using a Tescan Mira3 GMU. Stacked digital images were taken using a Leica Imaging System with a Z16 APO A microscope.

Morphological terms

Terms used for adult morphology follow Heraty et al. (2018). Terms used for larval morphology follow Heraty and Darling (1984), Darling and Miller (1991), and Darling (1992).

Molecular sequencing

Specimens were extracted using the DNeasy blood and tissue kit manufactured by Qiagen (Valencia, CA, USA) with 1 µL RNAse A added after incubation. Two gene regions were sequenced. The ribosomal gene 28S D2 used the following primers and thermocycler protocol: D2F (CGG GTT GCT TGA GAG TGC AGC) and D2Ra (CTC CTT GGT CCG TGT TTC); initial denaturization: 94 °C 3 min; (denaturization: 94 °C 1 min; annealing: 55 °C 1 min; extension: 72 °C 1 min) ×34; final extension: 75 °C 7 min. The mitochondrial gene COI-barcoding (COI-BC) used the following primers and thermocycler protocol: LCO1490 (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO2198 (TAA ACT TCA GGG TGA CCA AAA AAT CA); initial denaturization: 93 °C 3 min; (denaturization: 93 °C 15 sec; annealing: 46 °C 45 sec; extension: 68 °C 45 sec) ×34; final extension: 68 °C 7 min. PCR products were purified with DNA Clean & Concentrator™ -5 kits by Zymo Research (Irvine, CA, USA). PCR product concentrations were measured using Nanodrop 2000c (Thermo Scientific™). Each gene was PCR amplified individually and Sanger sequenced using both primers. Samples for Sanger sequencing were sent to Retrogen Inc. (San Diego, CA, USA) for sequencing on an Applied Biosystems 3730xl DNA Analyzer. Chromatograms were inspected for base calling errors and edited in Mesquite v.3.31 (Maddison and Maddison 2017b) using Chromaseq v.1.2 (Maddison and Maddison 2017a).

Molecular identification

We attempted to identify (or verify) specimens of parasitoid and host by comparing sequences with the online databases NCBI BLAST (Johnson et al. 2008) and BOLD (Ratnasingham and Hebert 2007) (Suppl. material 1: Tables S1, S2).

Morphological phylogenetics

We tested the placement of Eutrichosomatinae within the PLC by first using the planidial morphology data matrix developed by Heraty and Darling (1984) and later modified by Darling (1992). Within the PLC, the subfamilies of Perilampidae are paraphyletic in recent molecular studies (Munro et al. 2011; Heraty et al. 2013), and we treat Chrysolampinae, Perilampinae, and Philomidinae as separate terminal taxa and equivalent to Eucharitidae. We refined these previous character sets to include only informative and unambiguous characters at the family or subfamily level. Parsimony analyses were performed in PAUP* v4.0a (Swofford 2002) with default settings. We provide a detailed list of the characters, including character states, polarity inferences, and modifications from earlier studies (Heraty and Darling 1984; Darling 1992), with justifications for inclusion or exclusion of those characters.

Character 1. *Egg shape*: 0 = ovoid; 1 = stalked. Stalked eggs were regarded by Heraty and Darling (1984) as a synapomorphy for Eucharitidae, however at least three genera within Eucharitidae (*Indosema*, *Orasemorpha*, and *Timioderus*) lack egg stalks (Heraty 1994), and Chrysolampinae (Darling and Miller 1991), Philomidinae (Heraty et al. 2019), and Eutrichosomatinae all have egg stalks. The polarity cannot be inferred because the presence of egg stalks seems to be widespread throughout Chalcidoidea (e.g. *Aphelinus*, *Leucospis*, *Tetrastichus*, *Torymus*, and many more (Parker 1924)). We excluded this character from the analysis.

Character 2. *Egg sculpture*: 0 = smooth; 1 = ridged. Smooth eggs were inferred to be plesiomorphic by Heraty and Darling (1984), with ridged eggs only appearing in Perilampinae. This character is an autapomorphy for Perilampinae, thus excluded.

Character 3. *Sclerotization of terga* (=*tergites*): 0 = absent; 1 = present. Heraty and Darling (1984) treated this character as a combination of sclerotization and distinctiveness of terga, which was present in all of our ingroup taxa. Darling (1992) made this into two distinct characters: character 3 was sclerotization and character 3' was the shape of the terga (0 = completely encircling body; 1 = incomplete ventrad). All ingroup taxa were coded as sclerotized, and all ingroup taxa except Chrysolampinae and Eutrichosomatinae were coded as incomplete ventrad. The interpretation that Chrysolampinae and Eutrichosomatinae are more sclerotized than outgroup taxa with the terga completely encircling the body seems to be questionable, so we did not include the sclerotization character (3) but only used the shape character (3'), which is treated here as character 20.

Character 4. *Setal pattern of tergum III* (*TIII*): 0 = ventral setae absent; 1 = present. Heraty and Darling (1984) treated this character as a loss of the ventral setae in

Chrysolampinae and Oraseminae (Eucharitidae). The ventral pair of setae are difficult to define by position and are more accurately coded as the third pair of setae on TIII that are located ventrolaterally. Having the seta located on the ventrolateral margin is an autapomorphy of Eucharitidae, although it is not found in all taxa (Heraty and Barber 1990). However, these ventrolateral setae do appear to be present in both Chrysolampinae (Darling and Miller 1991) and Eutrichosomatinae, rendering this character uninformative for this analysis, thus it was excluded.

Character 5. *Distribution of dorsal setae*: 0 = absent; 1 = setae present on TI–III, V, VII, IX, XI; 2 = setae present on TI–III, V, VII, IX; 3 = setae present on TI–III, V. Heraty and Darling (1984) and Darling (1992) treated the absence of setae as the plesiomorphic condition, which is not easily justified given the widespread presence of setae in chalcidoid first-instar larvae (e.g. *Leucospis*, *Torymus*, *Eupelmus*, *Eurytoma* (Parker 1924)). For this analysis, the only informative dorsal seta is on TX. We excluded character 5, but added the presence of a seta on TX as character 21.

Character 6. *Dorsal fusion of terga I and II* (*TI and TII*): 0 = absent; 1 = present. Heraty and Darling (1984) treated this as a synapomorphy of Eucharitidae, however Oraseminae lack this fusion, and *Monacon* and *Krombeinius* (Perilampinae) have the terga fused (Darling 1995; Darling and Roberts 1999). This character is uninformative for this analysis and therefore excluded.

Character 7. *Ventral spines*: 0 = absent; 1 = present. Heraty and Darling (1984) noted that ventral spines are present in many ectoparasitic chalcidoid larvae. Spines of any kind are absent in Eucharitidae, and because Perilampidae are polymorphic, they interpreted the absence of spines as a synapomorphy of these two families. Darling (1992) combined character 7 (spines) with character 8 (tubercles) using the states: 0 = absent; 1 = tubercles present; 2 = spicules (spines) present. This is problematic because multiple terms have been applied to these spines (e.g. the ventral spines in *Chrysolampus* have been referred to as tubercles, pustules, and spicules without any clear distinction (Darling and Miller 1991)). Additionally, Chrysolampinae and Philomidinae were coded as having spicules (but not tubercles), and Perilampinae are coded as having tubercles (but not spicules) despite contradicting descriptions (cf. Darling and Miller 1991). We treat spines as a separate character from tubercles. Spines, represented as hook-like structures on the ventral region of the body segments and located between the tergal margins (cf. figs 17, 34, 37 in Heraty and Darling 1984), are a feature found only in some Perilampinae. They are uninformative for this analysis and excluded.

Character 8. *Lateral tubercles*: 0 = absent; 1 = present. Eutrichosomatinae have a series of tubercles across the ventral region of body segments II–XII (Fig. 1B, D, F; *tbs*). Heraty and Darling (1984) treated this as an autapomorphy of Chrysolampinae based on the description by Askew (1980). This character was further explored and illustrated on two species of *Chrysolampus* by Darling and Miller (1991), occurring on body segments II–XII. A similar patch of tubercles was found on the ventral region of body segment I in Philomidinae (Darling 1992). We excluded this character because of the difficulty assessing the homology of the various types of ventral and lateral protuberances.

Character 9. *Spiracles*: 0 = spiracles on TII, IV, V, VI; 1 = spiracles on TII; 2 = absent. Heraty and Darling (1984) noted that the plesiomorphic condition in Chalcidoidea is having pairs of spiracles on TII, IV, V, and VI, which is the state found in Eutrichosomatinae and several outgroup taxa (Parker 1924). Perilampinae and Philomidinae have lost all spiracles except on TII, and Eucharitidae and Chrysolampinae lack spiracles entirely.

Character 10. *Tergopleural line*: 0 = absent; 1 = present. This longitudinal line of thin, unpigmented cuticle going through the lateral sides of TII–IX is found in most Eucharitidae. It is absent in most Oraseminae, although present in *Orasemorpha* (Heraty 2000). In Eucharitinae, it is absent only in *Pseudochalcura* (Heraty and Barber 1990). It is present in Gollumiellinae, which is sister to Oraseminae + Eucharitinae (Heraty 2004), hence it is interpreted as a synapomorphy of Eucharitidae. It is uninformative for this analysis and was excluded.

Character 11. *Caudal cerci*: 0 = present as undifferentiated setae; 1 = absent; 2 = present as differentiated (longer and/or stouter) setae. These structures are defined as setae arising on the dorsum of TXII (Heraty and Darling 1984), which are larger than the setae on other body segments and often prolonged as stout spines or long hairs. If this character is treated as a synapomorphy of Eucharitidae and Perilampidae, the definition is not adequate because several groups have short setae (e.g. *Gollumiella longipetiolata* Hedqvist (Heraty 2004), *Hydrorhoa stevensoni* (Risbec) (Heraty 2002), *Australosema valgius* (Walker) and *Orasemorpha didentata* (Girault) (Heraty 2000)) and some have other setae on the body as long as the setae on TXII (e.g. *Perilampus chrysopae* Crawford (Clancy 1946), *Steffanolampus salicetum* (Steffan) (Darling 1999)). Eutrichosomatinae and Philomidinae both have short socketed setae on TXII and TXIII that are located more laterally (Eutrichosomatinae; Fig. 1H) or ventrally (Philomidinae). These setae appear to be homologous with cerci but without the enlargement and proposed specialized locomotion functions associated with Perilampinae and Eucharitidae (e.g. providing support to hold the body in an upright posture); therefore, we created another character state (state 0) for these taxa. Only Chrysolampinae has entirely lost setae on TXII. The position of the cerci on Eucharitidae appears to be between TXII and TXIII, which is a synapomorphy for the family but uninformative for this analysis.

Character 12. *Caudal pad*: 0 = absent; 1 = present. The last segment (TXIII) is membranous and expanded in Eucharitidae and Perilampinae to adhere to surfaces (Heraty and Darling 1984). However, the morphology does not appear to be different from other taxa in the PLC. We also think that polarity (absent ancestrally) cannot be assumed for this character because multiple other chalcidoid taxa have differentiated posterior segments with "suckers" or other ornamentations (e.g. *Leucospis* (Parker 1924)). This was excluded from the analysis.

Character 13. *Antenna*: 0 = present; 1 = reduced; 2 = absent. The short, conical, papilliform antenna (state 0) is considered plesiomorphic (Heraty and Darling 1984). These are present in Eutrichosomatinae and Chrysolampinae and are widespread across Chalcidoidea (e.g. *Leucospis*, *Torymus*, *Eupelmus*, *Eurytoma*, and more (Parker 1924)). Philomidinae have low, broad swellings that may indicate reduced antennae (Darling 1992).

Character 14. *Cranial setae*: 0 = present; 1 = absent. It is noted by Darling (1992) that the distinction and homology between cranial setae and cranial spines is not clear, which resulted in a combination of characters 14 and 15 with the states: 0 = 3–4 pairs; 1 = reduced number. Philomidinae was the only taxon coded as having complete cranial setae, however, all Perilampinae have 3 pairs of setae on the cranium. Because of the difficulty assessing polarity and homology for this character, it has been excluded.

Character 15. *Cranial spines*: 0 = present; 1 = absent. Some species of *Perilampus* have stout, recurved spines (Heraty and Darling 1984), which may be derived setae. This character is uninformative and excluded from this analysis.

Character 16. *Prelabium*: 0 = membranous; 1 = with sclerotized marginal rim. The prelabium is a depressed area with a sclerotized marginal rim and labial palpi on the margins (Heraty and Darling 1984). Presence of the labial sclerite was considered a synapomorphy for Eucharitidae and Perilampidae and is most easily identified when the palpi appear within or on the marginal rim. In Philomidinae, the labial palpi are not associated with a sclerite, which is thought to be the plesiomorphic condition (Darling 1992). This structure is extremely minute and difficult to score for most slide-mounted larvae. It is excluded from this analysis because the structure could not be verified in many of the taxa, although it is clearly present in *Eutrichosoma* (Fig. 1B, D, E, G; *prl*).

Character 17. *Postlabium*: 0 = non-eversible; 1 = enlarged and eversible. In Eucharitidae and Perilampinae, the postlabium is an eversible membranous sac surrounding the prelabium (Heraty and Darling 1984). This may be the same in Philomidinae (Darling 1992). Observations of specimens with everted postlabia are the only accurate way to code this character, and we could not observe this in Eutrichosomatinae, therefore it was excluded.

Character 18. *Labial plates*: 0 = absent; 1 = present. These are two sclerites found posterior to the prelabium in Eucharitidae (absent in Oraseminae). This character is uninformative for this analysis and excluded.

Character 19. *Pleurostomal setae*: 0 = present; 1 = spine-like; 2 = fused spines. These are setae lateral to the mouth, and they are present in most chalcidoid taxa (Heraty and Darling 1984). These setae do not appear to be modified in Eutrichosomatinae, Chrysolampinae, Philomidinae, or most Perilampinae, despite being previously coded as spine-like (Darling 1992). In Eucharitidae this has been coded as fused spines, but what was observed was not a socketed seta but instead a pointed projection of the cranial cuticle. This character was excluded from the analysis.

Character 20. *Shape of terga*: 0 = completely encircling body; 1 = incomplete ventrad. This is character 3' from Darling (1992). Terga encircling the body is seen in all other known chalcidoid first-instar larvae (Parker 1924) and is considered plesiomorphic for the PLC. Philomidinae, Perilampinae, and Eucharitidae all have incomplete terga. One exception is in the genus *Monacon* (Perilampinae), where the posterior terga (VII–XI) are ventrally fused, while the anterior terga are not (Darling and Roberts 1999). This could be treated as a third character state for a polymorphic coding of Perilampinae, but it would not have an impact on the relationships in this analysis.

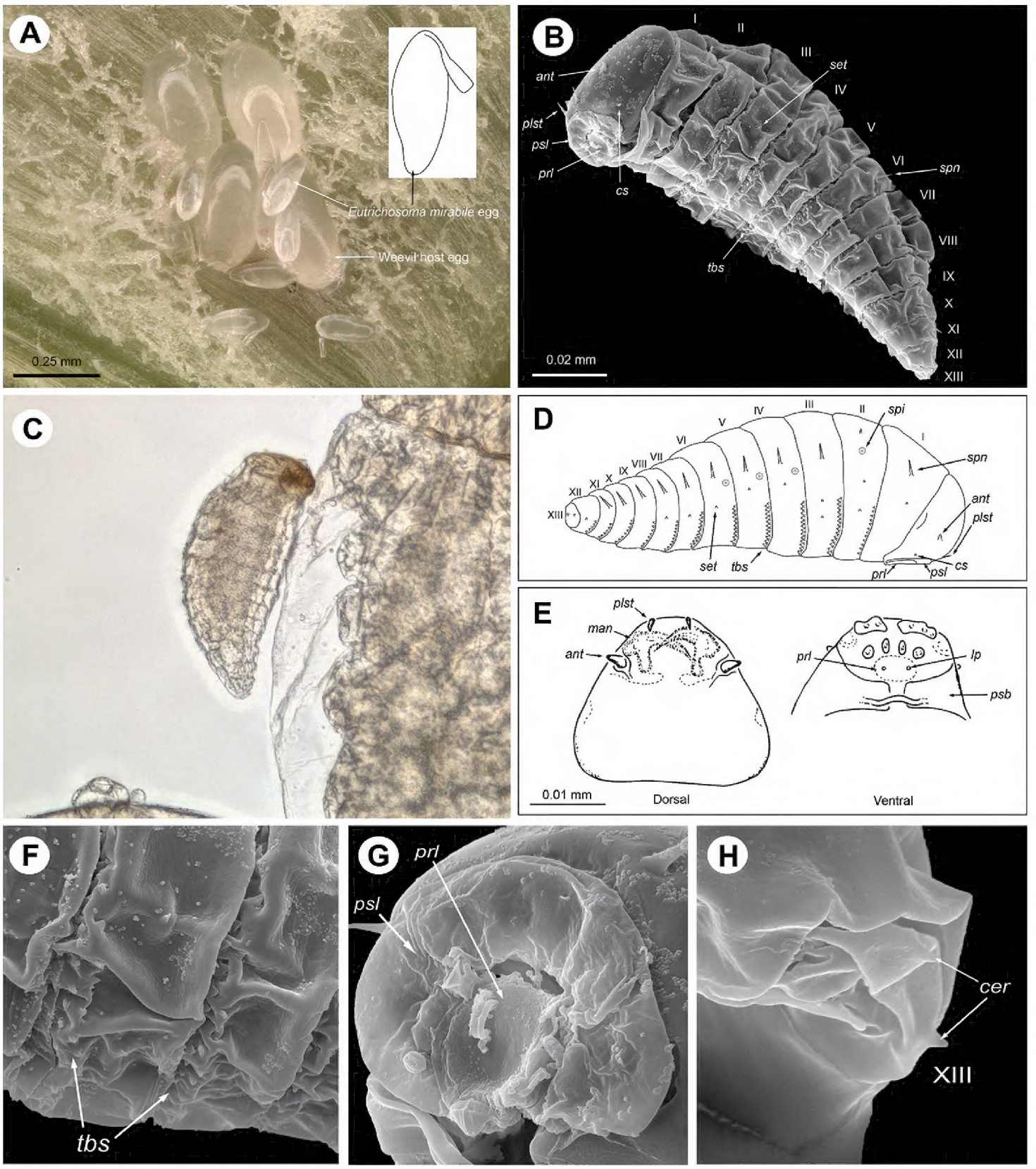


Figure 1. *Eutrichosoma mirabile* immature stages. **A** Eggs of *Eutrichosoma mirabile* (small, stalked) laid on top of the eggs of their weevil host (large, unstalked) within a seed pod of *Vachellia constricta*; inset: *Eutrichosoma mirabile* egg **B** SEM ventrolateral habitus image of a planidium of *Eutrichosoma mirabile* **C** planidium attached to host weevil larva **D** setal map of a *Eutrichosoma mirabile* planidium, modified from an illustration by Darling & Miller (1991) **E** head capsule of planidium, dorsal and ventral views **F** planidium TIII–IV, ventral tubercles **G** head, anterolateral view, showing the labial structure **H** TXIII with cerci, dorsolateral view. Abbreviations: *ant* = antenna, *cer* = cerci, *cs* = cranial spine, *lp* = labial palp, *man* = mandible, *plst* = pleurostomal seta, *prl* = prelabium, *psb* = pleurostomal bridge, *psl* = postlabium, *set* = seta, *spi* = spiracle, *spn* = spine, *tbs* = tubercles, I–XIII = terga numbered from anterior to posterior.

Character 21 (new). *Seta on tergum X:* 0 = present; 1 = absent. Setae present on all terga is treated here as the plesiomorphic state for the PLC based on the presence in many other chalcidoid taxa. Most taxa in the PLC have lost setae on TX, with the exception of Eutrichosomatinae, which maintains the ground plan configuration. We chose to focus on TX because the presence of setae on other terga are either present in all taxa (TI–III, V), only lost in Eucharitidae (TVII, IX), or require interpretation of the dorsal/lateral/ventral homology of the multiple setae present (TIV, VI, VIII).

Character 22 (new). *Seta on tergum XI*: 0 = present; 1 = absent. Loss of setae on TXI is a synapomorphy of Eucharitidae and Perilampinae.

Character 23 (new). *Behavior*: 0 = not ectoparasitic koinobiont; 1 = ectoparasitic koinobiont. All members of the PLC are ectoparasitic koinobionts. There are several examples of planidia residing internally (e.g. *Perilampus* spp.) or transdermally (e.g. *Orasema* spp.) within a host, but they always emerge to an external position to continue development through later instars; therefore, we do not consider these taxa endoparasitic sensu Heraty and Murray (2013). No other chalcidoids (where behavior is known) are ectoparasitic koinobionts because of the unique challenge of reattaching to the host after each molt, which requires increased mobility and host-recognition.

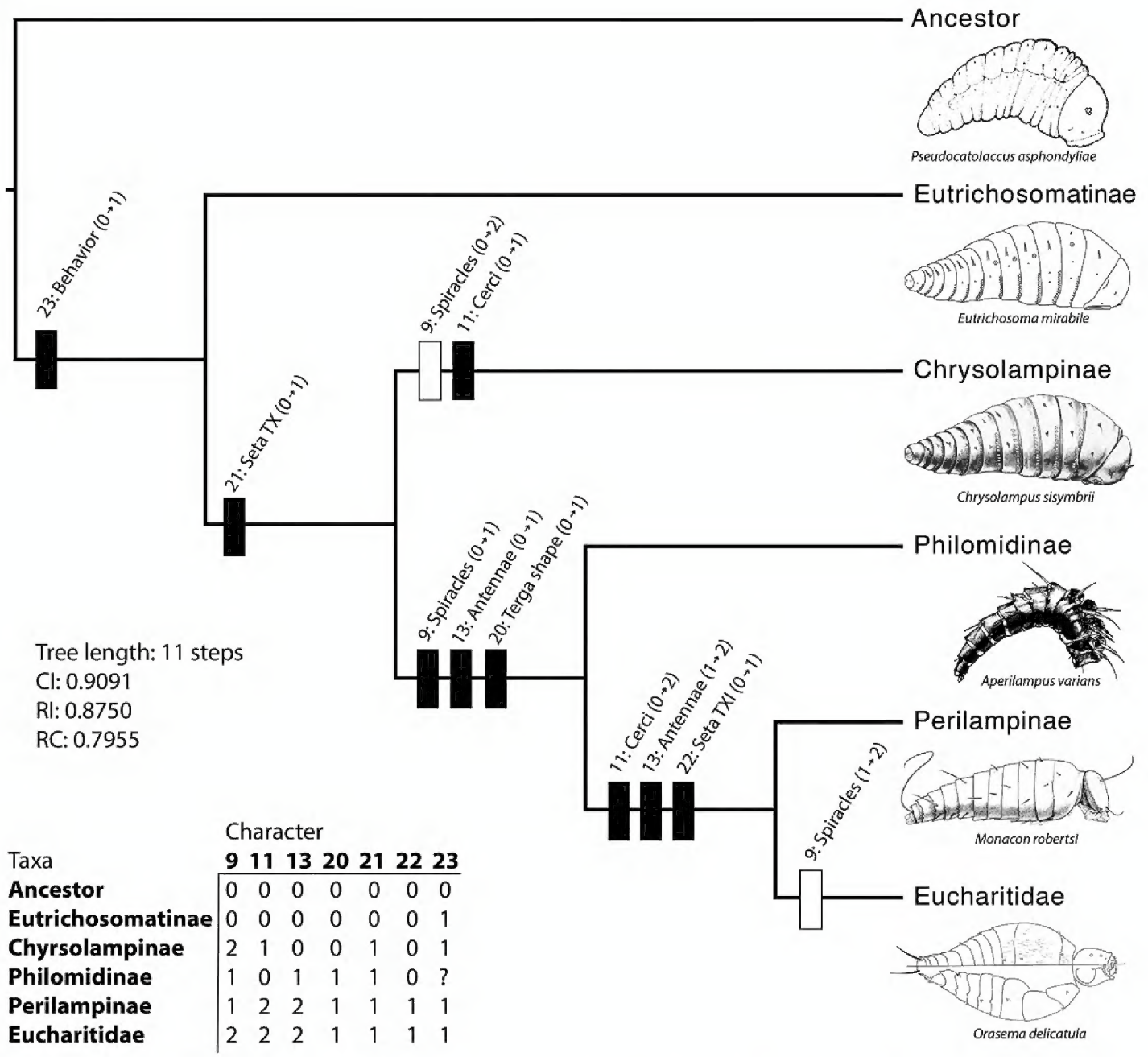
Results

Morphological phylogenetics of planidia

Our dataset was reduced to seven characters (9, 11, 13, 20–23) for the parsimony analysis. We chose to discuss all of the characters previously used because they can be valuable for future phylogenetic analyses at different levels (e.g. the genera of Eucharitidae). The major limitation for this analysis was finding informative characters with minimal ambiguity in the interpretation of their homology, which can be difficult for groups with simple morphology and large evolutionary gaps between sampled taxa. The most parsimonious tree was 11 steps and included only one homoplastic character (Fig. 2). We recovered the same topology as Heraty and Darling (1984) and Darling (1992) with the addition of Eutrichosomatinae, which was placed as the most plesiomorphic member of the PLC.

Eutrichosoma adult generic diagnosis

The inclusion of the new species of *Eutrichosoma* has modified the generic diagnosis (Bouček 1974). Margin of clypeus without incision; advanced occipital ridge directly posterior to the ocelli present or absent; the anterior transverse carina on the pronotal collar present or absent; mesoscutal midlobe completely separating the axillae medially, posteriorly reaching the mesoscutellum; fore wing without marginal fringe; postmarginal vein on the fore wing present, rudimentary (difficult to distinguish), or



Taxa	Character 9	11	13	20	21	22	23
Ancestor	0	0	0	0	0	0	0
Eutrichosomatinae	0	0	0	0	0	0	1
Chyrsolampinae	2	1	0	0	1	0	1
Philomidinae	1	0	1	1	1	0	?
Perilampinae	1	2	2	1	1	1	1
Eucharitidae	2	2	2	1	1	1	1

Figure 2. Most parsimonious tree from larval morphology, PAUP* analysis. Character state changes on branches are indicated by black bars (synapomorphies) and white bars (homoplasies). Character state matrix and tree statistics included. *Pseudocatolaccus asphondyliae* is shown as an example of a generic hymenopteriform larva with morphology that fits a hypothetical ancestor to the PLC. Illustrations of *Pseudocatolaccus asphondyliae* modified from Parker (1924); *Chrysolampus sisymbrii* modified from Darling and Miller (1991); *Aperilampus varians* modified from Darling (1992); *Moncacon robertsi* modified from Darling and Roberts (1999); *Orasema delicatula* modified from Burks et al. (2015).

absent. It differs from *Peckianus* by the iridescent blue or green coloration of the body, posterior end of the mesoscutal midlobe broadly separating the axillae (by 0.18–0.23× the length of the mesoscutal midlobe; 0.08–0.09× in *Peckianus*), marginal vein on fore wing short (0.1–0.13× the length of the fore wing; 0.2–0.21× in *Peckianus*). It differs from *Collessina* by having a moderately setose body (setae sometimes spatulate), head and mesosoma more coarsely reticulate, antenna with two anelli, scape not reaching median ocellus, notauli and scutoscutellar sulcus distinct, marginal vein on fore wing with a continuous width (not increasing distally).

Key to the species of *Eutrichosoma*

1 Body covered with distinctly wide, spatulate setae; stigmal vein angulate medially.....***Eutrichosoma mirabile* Ashmead**

– Body covered with thin, simple setae; stigmal vein nearly straight, without obvious angle.....**2**

2 Occipital carina present; mesoscutellar disc finely granulate; male antenna flabellate (females unknown); body length 2.9–3.1 mm.....
.....***Eutrichosoma flabellatum* Bouček**

– Occipital carina absent; mesoscutellar disc transversely imbricate; female antenna simple (males unknown); body length 1.9 mm... ***Eutrichosoma burksi* sp. nov.**

***Eutrichosoma mirabile* Ashmead**
Fig. 1A–H

Eutrichosoma mirabile Ashmead 1904: 375. Lectotype designated by Gahan and Peck (1946: 315): USA: Montana: Helena (female). Deposited in USNM.

Eutrichosoma albipes Crawford 1908: 158–159. Synonymy by Bouček, 1975. Holotype: USA: Texas: Dallas (female). Deposited in USNM.

E. mirabile; Bouček 1975: 132–133. Redescription and identification key.

Biology and life history. Eggs and first-instar larvae were found inside the early (green) seedpods of *Vachellia constricta* and associated with the presence of weevil eggs and larvae (Curculionidae). *Eutrichosoma mirabile* eggs are laid among the host eggs inside the seedpods between the ovule and the inner wall of the pod. Hatching of the parasitoid seems to coincide with or precede hatching of the host because parasitoid eggs were never observed without host eggs. The majority of planidia found were parasitizing first- or second-instar weevils (~85%). Typically, only one planidium was found per host, positioned anterodorsally on the body just behind the head attached by the mandibles; always on the external surface and never penetrating the cuticle. The remaining unattached planidia were observed crawling around near clusters of host eggs. Eggs and planidia were the only life stages of the wasps observed in the seedpods. While there may be several eggs and early instars of weevil (up to ~10) per ovule within a seedpod, by the time the weevils are in their fourth instar, there is only one individual per ovule remaining. Considering the wasps are ectoparasitic koinobionts, they are likely detaching then reattaching and repositioning themselves on their hosts between host molts or transferring between individual host larvae. Given the similarities between *E. mirabile* and chrysolampines (discussed below), it is assumed that the *E. mirabile* planidia remain attached externally to the weevil when it leaves the seedpod to pupate in the soil, where the parasitoid likely finishes development. We were not able to keep the weevil larvae alive outside of the pods to allow the parasitoid to develop further. Parasitism rates shown in Suppl. material 1: Table S3.

Egg (Fig. 1A). Egg body length approximately 0.2 mm, maximum width approximately 0.07 mm; ovoid; caudal stalk about half as long as the body of the egg. Eggs separate, not forming tight clusters.

Planidium (Fig. 1B–H). Length approximately 0.13 mm, maximum width approximately 0.05 mm; fusiform in shape. Body and cranium white, darkened around mouth (Fig. 1C). Cranium with one pair of short, papilliform antennae (*ant*), one pair of longer, thinner pleurostomal setae (*plst*), and one pair of minute, lateral cranial spines (*cs*); postlabium (*psl*) large, flat, circular, and surrounding prelabium (*prl*); labial palp (*lp*) present; pleurostomal bridge (*psb*) present and connected by thin integument (Fig. 1E). Thirteen body segments beyond head; terga lightly sclerotized and ring-like, encircling the body; band of 1–2 irregular rows of tubercles (*tbs*) on anteroventral side of terga II–XII; prominent dorsolateral spines (*spn*) on terga I and III–XI; setae (*set*) present on terga I–VIII and XII, with three pairs on tergum II and two pairs on tergum III; short cerci present on XIII (*cer*); spiracles on terga II, IV–VI (*spi*) (Fig. 1B, D, F–G).

Determining if a first-instar larva is a type I planidium (i.e. undergoes hypermetamorphosis sensu Pinto (2009)) requires examination of subsequent instars, which we did not find for *Eutrichosoma*. However, the mobility of the larvae observed in the seedpods and inferred from their koinobiont ectoparasitc behavior suggests that *Eutrichosoma* behavior is congruent with other PLC larvae, even if their morphology is not as derived as Eucharitidae, Perilampinae, or Philomidinae, which are all more heavily sclerotized and generally lack ventral fusion of the terga. Larvae of Eutrichosomatinae and Chrysolampinae appear to be somewhat intermediate between typically hymenopteriform first instars of other chalcidoid taxa and the highly derived planidial larvae in the PLC.

Material examined. USA: Arizona: Cochise Co.: Canadian Lane, Portal, 1426m, 31°55'1"N, 109°07'37"W, 28.viii.2016, A. Baker & S. Heacox, **AB16.024** [2 larvae slide mounted, UCRCENT00513221–2]; 4.viii.2018, A. Baker, S. Heacox, L. Kresslein, **AB18.007** [larvae in alcohol, UCRCENT00513223] **deposition UCRC**.

***Eutrichosoma burksi* sp. nov.**
http://zoobank.org/A3F880D1-467D-4201-A113-E39BE80A644F
Fig. 3A–F

Diagnosis. Recognized from other *Eutrichosoma* by the following combination of characters: body with metallic green coloration; stigma enlarged, stigmal vein short and not elbowed; setae relatively thin and sparse; transversely imbricate sculpture on mesosoma dorsally; lacking vertexal carina.

Female. Length 1.9 mm.

Color. Head, mesosoma, scape, pedicel, and coxae dark green; anellus and flagellum brown; mandible reddish brown; maxilla and labium brown. Femora and tibiae dark brown with green reflections medially, pale at tips. Fore wing hyaline, venation pale brown. Gaster dark brown with green iridescence.

Head (Fig. 3B). Head in frontal view subcircular; head width:height 1.24; face reticulate; scrobal depression shallow, laterally rounded; eyes with minute setae; malar

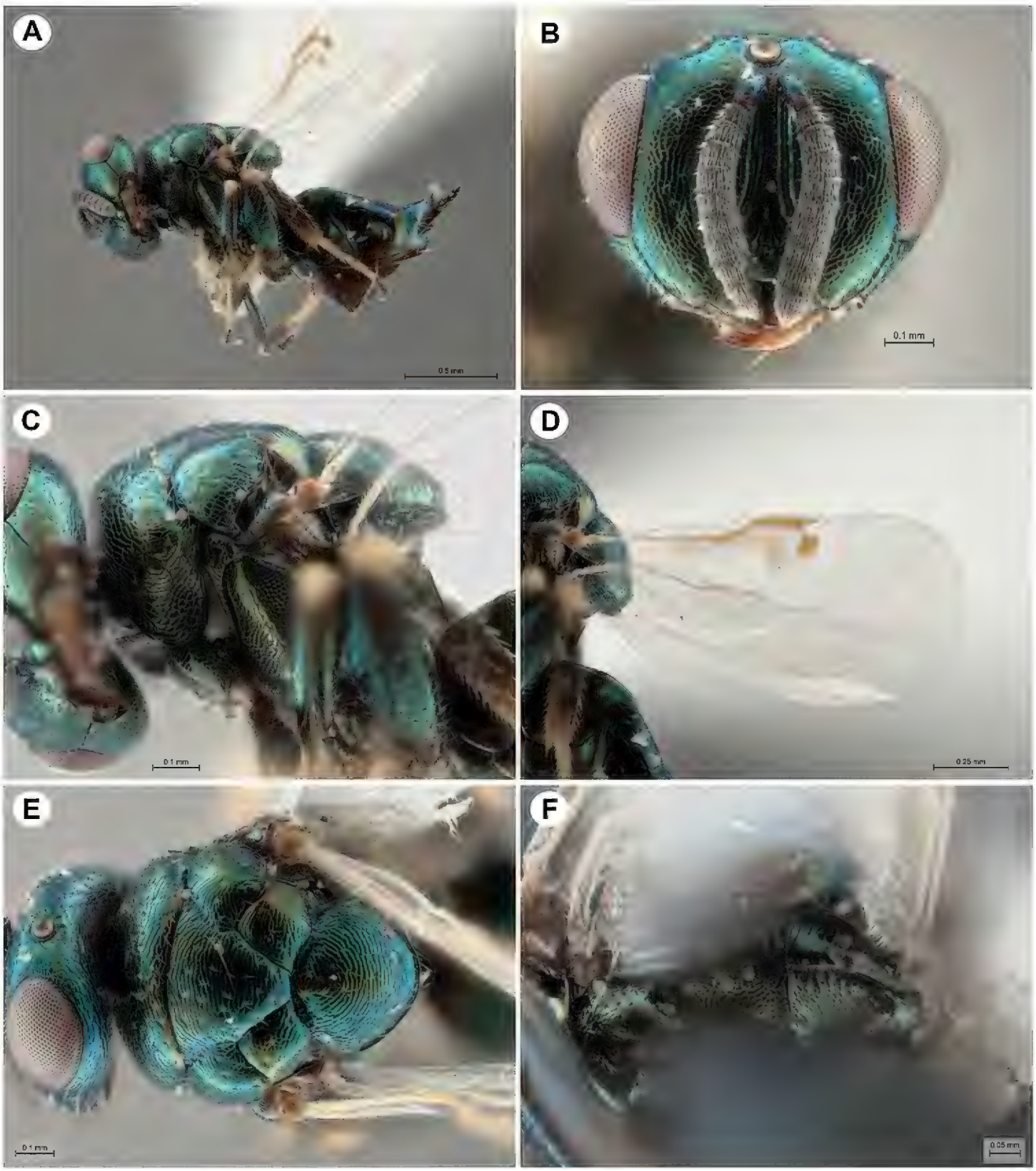


Figure 3. *Eutrichosoma burksi* sp. nov. holotype, adult female. **A** Lateral habitus **B** anterior head **C** lateral mesosoma **D** lateral wing **E** dorsal mesosoma **F** posterior propodeum.

sulcus present; clypeus smooth with rounded margin; epistomal sulcus distinct and sharply defined; anterior tentorial pit shallow; anteclypeus distinct, broadly rounded; palpal formula 4:3; mandibular formula not observed; occiput strigate, emarginate in dorsal view, dorsal margin evenly rounded; temples present, rounded. Antennal scape not reaching median ocellus; pedicel elongate, more than 1.5× as long as broad; antenna with 12 flagellomeres, including small terminal button (F12) at end of clava (clava 4-segmented); flagellum length:head height 0.81; anellus disc-shaped; second flagellomere (F2) 0.78× as long as broad, 0.75× as long as F3; following flagellomeres subequal in length, gradually broader; clava subconical.

Mesosoma (Fig. 3C–F). Mesosoma length:height 1.28; mesoscutal midlobe, lateral lobe, axilla, and mesoscutellum transversely imbricate to coriacious, sparsely setose (Fig. 3E); notauli deeply impressed along entire length; axilla dorsally rounded, on roughly same plane as mesoscutellar disc; scutoscutellar sulcus broad, irregularly foveate, fused with transscutal articulation medially; propodeal disc broadly rounded, reticulate, with median carina (Fig. 3F); callus bulbous, projecting posteriorly beyond the lateral margins of the propodeum, reticulate, with several long hairs; mesepisternum reticulate; upper mesepimeron smooth; lower mesepimeron reticulate; transepimeral sulcus distinct; propleuron nearly flat, transversely imbricate. Hind femur 3.39× as long as broad, with long stout setae; hind tibia with long stout setae. Fore wing 2.13× as long as broad, basal cell and speculum bare, costal cell sparsely setose, wing disc moderately setose; marginal fringe absent; submarginal vein with nine long setae dorsally; marginal vein with eight long setae along the margin; parastigmal vein slightly thicker than submarginal, constricted at connection with marginal vein; stigmal vein straight, narrow, short; stigma large, slightly angled; uncus absent; postmarginal vein short but obvious; hind wing costal cell with a broad bare area medially.

Metasoma. Gaster appears sessile, petiole short and indistinct; first gastral tergum longer than subsequent terga; sparsely setose dorsally, with more setae laterally. Ovipositor sheaths protruding a short distance past the last gastral tergum.

Male. Unknown.

Biology. Unknown

Material examined. ***Holotype*: USA: California:** San Bernardino Co.: Kelso Dunes Rd, 775m, 34°53'23"N, 115°43'05"W, 19.v.2001, D. Yanega [1 ♀, UCRCENT00221857], **deposition UCRC**.

Etymology. Named in honor of Roger A. Burks, whose expertise led to the recognition of this specimen as a new species.

Discussion

The larval morphology and life history of *Eutrichosoma mirabile* is quite similar to species of *Chrysolampus* (Chrysolampinae). Both taxa lay their eggs in seedpods infested with seed-feeding weevil larvae (Darling and Miller 1991). The larvae of these wasps are strikingly similar, with the position of the spines and setae being the most obvious means to differentiate the two. These observations along with the digitate labral morphology for adults (Darling 1988) and the molecular phylogenetic support from both traditional Sanger sequencing datasets (Heraty et al. 2013) and next-generation sequencing datasets (Heraty et al. unpublished; Rasplus et al. unpublished), make placement of Eutrichosomatinae in the PLC highly supported. The position of this subfamily within the PLC, however, is far less certain. While the planidia of *E. mirabile* may morphologically appear plesiomorphic to the rest of the PLC (Darling 1988), it is not consistently placed as sister to the remaining PLC with molecular data (Heraty et al. unpublished). Our analysis of a limited number of morphological characters of the

first-instar larvae supports the sister-group relationship between Eutrichosomatinae and the remaining PLC, but interpretations of larval character homologies at the family level are very difficult to make.

Pinto (2009) defines planidium as a legless type I hypermetamorphic first-instar larva; type I is characterized by the larva having to locate its own food source, survive for considerable time without desiccating or feeding, and being active, slender, and well-sclerotized. This definition accurately describes the larvae of Eucharitidae, Perilampinae, and Philomidinae, but it cannot be unambiguously applied to Chrysolampinae or Eutrichosomatinae. Females of these two taxa oviposit into seedpods, their larvae are not directly exposed to an external environment, and heavy sclerotization of the body segments is less necessary. As well, the enclosed environment does not necessitate the need for the various ornamentation of setal modifications of the other taxa that may be associated with extreme mobility. Highly mobile ectoparasitic first-instar larvae are known in other Chalcidoidea, but these are usually idiobionts where eggs are laid onto and develop on a single developmental stage of the host, with the larvae searching for an appropriate place to initiate feeding (i.e. *Spalangia* (Richardson 1913; Clausen 1940a)) or even using the first instar to cruise the host and kill competing sibling larvae before initiating feeding (i.e. *Leucospis* (Graenicher 1906; Clausen 1940a)). *Eutrichosoma mirabile* and *Chrysolampus* are unique in Chalcidoidea in that they are both ectoparasitic koinobionts, with the first instars not only highly mobile but able to pass through different life stages of the host, in this case from the host first instar to pupa. This combination is rare in Hymenoptera, being found in some Ichneumonidae, Braconidae, and Dryinidae (Gauld and Bolton 1988; Gauld and Hansen 1995; Quicke 1997). Some Eulophidae have been designated ectoparasitic koinobionts: *Eulophus larvarum* (L) (Shaw 1981) and *Euplectrus* sp. (Neser 1973); however, these taxa are fundamentally different than all of the other ectoparasitic koinobionts because they develop on a single instar of their host caterpillar, which will continue to feed for a time but does not continue developing and renders the parasitoids' status as koinobionts ambiguous. The first-instar larvae of Chrysolampinae and Eutrichosomatinae appear to represent the transition between idiobiont hymenopteriform larvae (limited mobility, weakly sclerotized) and koinobiont planidia (highly mobile, heavily sclerotized). We feel that the application of a combination of behavioral characters (koinobiont, ectoparasitoid, with eggs not directly placed on the host) allows inclusion of these transitional forms into what we term planidial larvae.

While the 28S-D2 gene region confirmed the identity of the parasitoid, which had an exact match between larva and adult (Suppl. material 1: Table S1), the identification of the larval host weevils remains a mystery. There were no exact matches between the sequenced larval weevils and any adult weevils that we collected from the acacia. Furthermore, the BLAST and BOLD results for the host larva were inconclusive past the family level (Curculionidae) for both gene regions, with the top hits being distant matches to multiple subfamilies, including Curculioninae, Ceutorhynchinae, and Tychiinae (Suppl. material 1: Table S2).

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Supplementary material 1

Tables S1–S3

Authors: Austin J. Baker, John M. Heraty

Data type: molecular data

Explanation note: **Table S1.** Parasitoid larva. Top hits from NCBI BLAST and BOLD online databases for two gene sequences (28S-D2 rDNA and COI-BC mtDNA) obtained from the parasitoid first-instar larva and confirming the identity as *Eutrichosoma mirabile*. **Table S2.** Host larva. Top hits from NCBI BLAST and BOLD online databases for two gene sequences (28S-D2 rDNA and COI-BC mtDNA) obtained from the host larva and confirming the identity as Curculionidae but leaving the subfamily, genus, and species unconfirmed. **Table S3.** Parasitism rates. Summary data from two collecting trips to southeastern Arizona (August 2016 and 2018) showing the rates of parasitism for the host and parasitoid, eggs and larvae on whitethorn acacia (*Vachellia constricta*).

Link: https://doi.org/10.3897/jhr.75.47880.suppl1